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(71) Applicant (for all designated States except US):
GILTECH LIMITED [GB/GB]; 9/12 North Harbour Estate, Ayr KA8 8BN (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): HEALY, David, Michael [IE/GB]; Midton House; By Alloway, Ayr KA7 4EG (GB). GILCHRIST, Thomas [GB/GB]; 67 Midton Road, Ayr KA7 2TW (GB).

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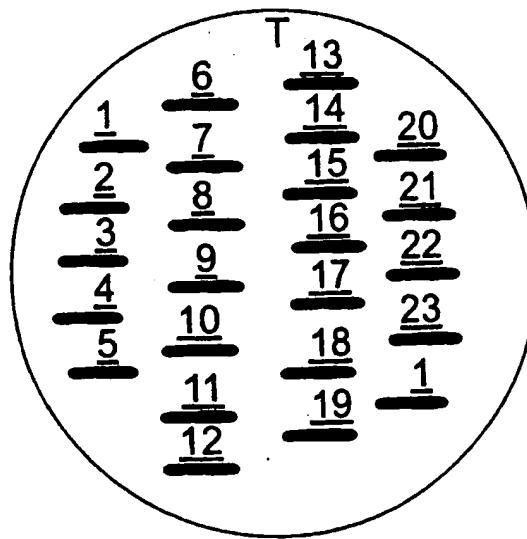
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(54) Title: SUTURE MATERIAL



(57) Abstract: A surgical suture material having either an external surface at least partially coated with an anti-microbial composition or an anti-microbial agent incorporated therein. Preferably the anti-microbial agent is a water-soluble metal ion-releasing glass in particle form.

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1 SUTURE MATERIAL

2

3 The present invention relates to a suture material
4 having antimicrobial characteristics.

5

6 Sutures are the threads or wires used to stitch two
7 bodily surfaces together. Typically, sutures are
8 required to close surgical incisions and to treat deep
9 lacerations inflicted on a patient.

10

11 Suture types fall into two main categories; absorbable
12 and non-absorbable. Additionally, the sutures can be
13 of monofilament or multifilament structure, with the
14 multifilament sutures being braided or twisted. A
15 variety of sizes of sutures are available. Typical
16 commercially available suture types are listed below:

17

18 Non-absorbable:

19

20 Silk twisted, braided & multifilament
21 Nylon polyamide monofilament

1 Polypropylene monofilament
2 Polyester braided multifilament
3 PTFE monofilament
4 PVDF monofilament
5 Stainless steel monofilament
6 Linen multifilament
7

8 **Absorbable:**

9
10 PGA monofilament & multifilaments
11 PLA monofilament & multifilaments
12 Lactide/Glycolide
13 Copolymers monofilaments & multifilaments
14 Catgut monofilament
15 Collagen monofilament
16

17 In general braided multifilaments have a smoother
18 surface than the alternative twisted multifilaments and
19 so remain more cohesive when stitched. Monofilaments,
20 being formed from a single fibre, cannot unravel and
21 thus lose cohesiveness.

22
23 To improve the lubrication along the surface of the
24 suture and to provide friction to improve knot
25 strength, the sutures may be coated. Conventionally
26 however monofilament sutures are not coated. Coatings
27 which may be applied include 100% beeswax BP,
28 Silicone, PTFE (e.g. Teflon), PVP, polylactic acid
29 (PLA), polyglycolactide (PLG), polycaprolactones and
30 copolymers thereof. Often the coatings will

1 incorporate detergents or other lubricating substances,
2 e.g. calcium stearate.

3

4 However, sutures used for surgical wound closure are
5 associated with increased bacterial infectivity.

6 Sutures draw contaminants into the wound closure and
7 provide a surface along which micro-organisms can track
8 as a biofilm. Contamination of the wound via the
9 suture can arise from the local environment
10 (particularly in gut surgery), the closure area around
11 the wound, inappropriate handling of the suture or from
12 contaminated suture stock.

13

14 It is an object of the present invention to reduce the
15 risk of infection due to suturing a wound, by providing
16 sutures having antimicrobial characteristics.

17

18 Thus, in one aspect, the present invention provides a
19 surgical suture material having either:

20

21 a) an external surface at least partially coated with
22 an anti-microbial composition comprising an anti-
23 microbial agent; or

24

25 b) an anti-microbial agent incorporated therein.

26

27 The surgical suture material may be formed from any
28 suitable substance and may be absorbable or non-
29 absorbable. Mention may be made of silk, polyester,
30 nylon, polypropylene, polyvinylidenefluoride, linen,
31 steel wire, catgut (beef serosa or ovine submucosa),

1 polyglycolactide, polyamide (e.g. polyamide nylon),
2 fibroin, polyglycolic acid and copolymers thereof. The
3 sutures may be monofilament or may be braided or
4 twisted multifilament yarns.

5

6 The anti-microbial composition if to be applied as a
7 coating may be applied to the suture surface in the
8 same way as a conventional coating. Indeed, a
9 conventional coating material admixed with or including
10 an anti-microbial agent is suitable for use in the
11 present invention.

12

13 Preferably the anti-microbial agent is biodegradable
14 over a period of time compatible with the timescale of
15 wound healing. A slow-release of the anti-microbial
16 active ingredient of the agent over a period of weeks
17 or months is thus desirable.

18

19 A preferred anti-microbial agent is a water-soluble
20 metal ion-releasing glass, especially in particle (e.g.
21 fine powder) form that may be simply admixed with a
22 conventional coating and applied to the suture
23 material. Advantageously the metal released by the
24 glass is silver.

25

26 Thus we have found that by incorporating a comminuted
27 anti-microbial water soluble glass either into the
28 suture material itself or coated onto the external
29 surface thereof, the infectivity of a wound site is
30 reduced, whilst the handling characteristics
31 (knotability and insertion lubricity) are maintained.

1 Phosphorous pentoxide (P_2O_5) is preferably used as the
2 glass former of the biodegradable glass used in the
3 coating.

4

5 Generally the mole percentage of phosphorous pentoxide
6 in the glass composition is less than 85%, preferably
7 less than 60% and especially between 30-60%.

8

9 Alkali metals, alkaline earth metals and lanthanoid
10 oxides or carbonates are preferably used as glass
11 modifiers. Generally, the mole percentage of alkali
12 metals, alkaline earth metals and lanthanoid oxides or
13 carbonates is less than 60%, preferably between 40-60%.

14

15 Boron containing compounds (eg B_2O_3) are preferably used
16 as glass additives. Generally, the mole percentage of
17 boron containing compounds is less than 15% or less,
18 preferably less than 5%.

19

20 Other compounds may also be added to the glass to
21 modify its properties, for example SiO_2 , Al_2O_3 , SO_3 ,
22 sulphate ions (SO_4^{2-}), transition metal compounds (eg.
23 first row transition metal compounds) or mixtures
24 thereof.

25

26 Typically the soluble glasses used in this invention
27 comprise phosphorus pentoxide (P_2O_5) as the principal
28 glass-former, together with any one or more
29 glass-modifying non-toxic materials such as sodium
30 oxide (Na_2O), potassium oxide (K_2O), magnesium oxide
31 (MgO), zinc oxide (ZnO) and calcium oxide (CaO) or

1 mixtures thereof. The rate at which the glass
2 dissolves in fluids is determined by the glass
3 composition, generally by the ratio of glass-modifier
4 to glass-former and by the relative proportions of the
5 glass-modifiers in the glass. By suitable adjustment
6 of the glass composition, the dissolution rates in
7 water at 38°C ranging from substantially zero to
8 25mg/cm²/hour or more can be designed. However, the
9 most desirable dissolution rate R of the glass is
10 between 0.01 and 2.0mg/cm²/hour.

11

12 The water-soluble glass is preferably a phosphate
13 glass, and preferably comprises a source of silver ions
14 which may advantageously be introduced during
15 manufacture as silver orthophosphate (Ag₃PO₄). The
16 glass preferably enables controlled release of silver
17 or other metal ions, for example Zn, Cu, Mg, Ce, Mn,
18 Bi, Se, Cs and mixtures thereof (preferably Ag, Cu, Zn
19 and Mg and mixtures thereof) and other constituents in
20 the glass and the content of these additives can vary
21 in accordance with conditions of use and desired rates
22 of release, the content of silver generally being up to
23 5 mole %. While we are following convention in
24 describing the composition of the glass in terms of the
25 mole % of oxides, of halides and of sulphate ions, this
26 is not intended to imply that such chemical species are
27 present in the glass nor that they are used for the
28 batch for the preparation of the glass.

29

30 The optimum rate of release of the metal ions (eg Ag,
31 Cu, Zn or Mg, or any of the other metal ions mentioned

1 above) into an aqueous environment may be selected by
2 circumstances and particularly by the specific function
3 of the released metal ion. The invention provides a
4 means of delivering metal ions to an aqueous medium at
5 a rate which will maintain a concentration of metal
6 ions in said aqueous medium of not less than 0.01 parts
7 per million and not greater than 10 parts per million.
8 In some cases, the required rate of release may be such
9 that all of the metal added to the system is released
10 in a short period of hours or days and in other
11 applications it may be that the total metal be released
12 slowly at a substantially uniform rate over a period
13 extending to months or even years. In particular cases
14 there may be additional requirements, for example it
15 may be desirable that no residue remains after the
16 source of the metal ions is exhausted or, in other
17 cases, where the metal is made available it will be
18 desirable that any materials, other than the metal
19 itself, which are simultaneously released should be
20 physiologically harmless. In yet other cases, it may
21 be necessary to ensure that the pH of the resulting
22 solution does not fall outside defined limits.

23

24 Generally, the mole percentage of these additives in
25 the glass is less than 25%, preferably less than 10%.

26

27 In a preferred embodiment the biodegradable glass
28 comprises 20-35 mole% Na₂O; 18-30 mole% CaO and 45-60
29 mole% P₂O₅.

30

1 It is a further object of the invention to provide a
2 method of reducing the risk of infection and provide
3 faster and more efficient healing of the wound by using
4 the suture material of the invention to close the
5 wound.

6

7 The present invention will now be further described by
8 reference to the following, non-limiting, examples and
9 to figures, in which:

10

11 Fig. 1 : shows the template used in the example
12 to facilitate regular application of the
13 suture lengths on the plates.

14

15 Figs. 2-6 : show digitally generated photographic
16 images showing the results of Example 2.

17

18 EXAMPLE 1: Suture Coating Preparation

19

20 Glasses were prepared according to Table 1.

21

22

23

24

25

26

27

28

29

30

31

1 Table 1

2

Annealed Solution Rate Mg.cm. ⁻² hr ⁻¹	Mode μm	Composition				Code
		Na ₂ O	CaO	P ₂ O ₅	Ag ₂ O	
0.14	23.71	22	26.5	47.0	4.5	01
1.42	19.44	33.	16.5	47.0	3.0	02
0.27	19.96	27.5	22	47.0	3.5	03
1.42	6.50	33	16.5	47.0	3.0	04
16.05	14.02	30	10	47.5	6.5	05
6.02	12.64	36	13	47.5	3.5	06
3.48	25.44	34.5	14.5	47.5	3.5	07
11.28	12.20	36	11.5	47.5	5.0	08

3

4 These glasses were prepared as powders (mode size given
 5 in μm in Table 1 above) for incorporation into a suture
 6 coating.

7

8 Testing

9

10 Physical/Mechanical

11

12 It is important that addition of silver ion releasing
 13 glass into the coating does not compromise the physical
 14 or mechanical properties of the suture. The smoothness
 15 of the coating is essential in ensuring smooth
 16 insertion of the suture. The coating should not slough
 17 off on insertion and the knot properties should not be

1 reduced. Test samples show that up to 2.5% wt/wt
2 (final dry weight of coating) of glass powder could be
3 added to the coating without affecting these properties
4 and up to 5% wt/wt may be possible with some samples.

5

6 Samples

7

8 Glass samples 01 and 04 were applied to
9 glycolide/lactide copolymer braided multifilament
10 sutures in a glycolide/caprolactone coating at various
11 weights. The coat weight applied was 2% wt/wt dry
12 weight coating onto the suture. Samples G1 to G10
13 contain glass 01 from 0.25-2.5% wt/wt dry weight in the
14 coating. G11 to G20 contain glass 04 at 0.25 to 2.5%
15 wt/wt dry weight in the coating. G21 is a nylon
16 monofilament with 2% wt/wt coating containing 2.5%
17 wt/wt of 04. This coating did not bond well with the
18 suture G22 and G23 and control copolymer and control
19 nylon sutures respectively.

20

21 EXAMPLE 2: Anti-microbial Activity

22

23 G1 to G23 were screened against 17 test organisms.

24

25 Suture Material

26

27 G1 to G20-Violet Polysorb size 0 sutures

28 G21-Dacron suture size 2/0

29 G22-Violet Polysorb control

30 G23-Dacron control

31

1 Test Organisms

2

3 A panel of "wild-type" clinical isolates was used
4 except for organism 5, *Staph epidermidis* NCTC 11047.5 This organism is a reference organism noted to be
6 sensitive to test sutures utilised in a previous
7 experiment.

8

9 Gram-positive Isolates

10

11 1. *Enterococcus faecalis*12 2. *Staphylococcus aureus*13 3. *Enterococcus faecalis* - vancomycin resistant (VRE
14 - VanA genotype)15 4. Methicillin-resistant *Staphylococcus aureus* (MRSA
16 - epidemic type 15)17 5. *Staphylococcus epidermidis* NCTC 11047.18 6. *Streptococcus agalactiae* (Group B streptococcus)

19

20 Gram-negative Isolates

21

22 7. *Stenotrophomonas maltophilia* (formerly *Xanthomonas*
23 *maltophilia*)24 8. *Pseudomonas aeruginosa* - strain 125 9. *Pseudomonas aeruginosa* - strain 226 10. *Serratia marcescens*27 11. *Enterobacter cloacae*28 12. *Morganella morganii*29 13. *Escherichia coli*30 14. *Klebsiella pneumoniae*31 15. *Acinetobacter* sp.

1 Yeasts

2

3 16. *Candida albicans*

4 17. *Candida glabrata*

5

6 Method

7

8 *Media* - 9 cm plates of Oxoid Iso-sensitest agar were
9 used for all organisms except the candida isolates
10 which were plated on Yeast Morphology Agar.

11

12 *Inoculum* - Overnight plate cultures of the test
13 organisms were emulsified in physiological saline to
14 achieve a semi-confluent growth on the agar plates.

15

16 *Inoculum procedure* - The plates were pre-dried at 37°C
17 for 2 hours. The inoculum was applied using a sterile
18 swab using a cross-streaking technique.

19

20 *Suture application* - The suture was cut into
21 approximate 1 cm lengths using sterile instruments.
22 Where possible, straight sections of suture were used.
23 A template was constructed to facilitate regular
24 application of the suture lengths. Each plate of test
25 organism had the series of 21 test and 2 control
26 sutures applied, with a replicate of suture G1 as an
27 internal control on the far side of the plate (see
28 Figure 1 for template). Each suture was pressed down
29 with sterile forceps to optimise contact with the agar
30 surface.

31

1 Incubation - 37°C for 18 hours. The plates were
2 reassessed after a further 24 hours.

3

4 Recording of results - The maximum width of the zone of
5 inhibition at right angles to the suture length was
6 recorded to nearest 0.5 mm (the maximum width was
7 recorded to avoid skewing of results due to incomplete
8 contact of parts of the suture with the agar surface,
9 resulting in irregular zones - see photographic
10 results).

11

12 Results

13

14 See digitally generated photographic images provided as
15 Figs. 2 to 6 and Table 2.

16

17 Conclusions

18

19 G21-Dacron suture:

20 Zones of inhibition were seen with all test organisms
21 except *Candida albicans* (organism 16).

22

23 G23-Dacron control suture:

24 No demonstrable activity.

25

26 G1-G20-violet Polysorb suture:

27 There was a general trend towards increasing activity
28 with the higher Polysorb suture numbers, with zone
29 sizes plateauing with G14, 15 and 16 followed by a
30 slight decline.

31

1. Activity was seen against most organisms in the panel.
2. No zones were seen with two candida isolates (organisms
3. 16 and 17) and the zones for *Stenotrophomonas*
4. *malophilia* (organism 7) and *Enterobacter cloacae*
5. (organism 11) tended to be smaller, or absent compared
6. to the other Gram-negative isolates.

7

8. Activity against the staphylococcal isolates (organisms
9. 2, 4 and 5) was seen with virtually all sutures. This
10. is of note given the particular importance of
11. staphylococci in the aetiology of stitch abscesses.

12

13. The enterococci and streptococci (organisms 1, 3 and 6)
14. demonstrated the largest zones of inhibition.
15. Interestingly, the control suture (G22) also yielded
16. significant zones for all three organisms, indicating
17. that one of the constituents of the suture has
18. antimicrobial activity in its own right. This
19. constituent must be released from the suture and be
20. able to diffuse through the agar. There is apparent
21. interaction with the components of the test sutures -
22. G2 consistently gave zones smaller than the control.

23

24. As will be seen from the digital images (Figs. 2 to 6)
25. the inoculum ranged from semi-confluent to near
26. confluent growth. The Gram-negative organisms tended
27. to a heavier inoculum. Despite the significant
28. challenge, zones of inhibition were seen. At this
29. stage the duration of activity of the test sutures
30. cannot be stated - however, transient contact with the

1 surface of the agar (duration less than 5 seconds)
2 resulted in a small zone of inhibition.

3

4 EXAMPLE 3: Anti-microbial Activity

5

6 Protocol

7

8 As for Example 2.

9

10 The experiment was performed to confirm the results
11 from the previous experiment, in particular the
12 activity of the G22 control suture against the
13 enterococci and streptococci, and the effect of a lower
14 inoculum on the results from the Gram-negative
15 organisms.

16

17 Results

18

19 See Table 3.

20

21

22

23

24

25

26

27

28

29

30

31

1 Table 3 : Maximum width of zone of inhibition measured
 2 at right angles to the suture (millimetres)
 3

ORGANISM				
	1 Enterococcus	5 Staph 11047	6 Gp B strept	13 E Coli
Suture				
G4	9 m	2 m	8 m	0 m
G9	9 m	2.5 m	9 m	1 m
G11	8 m	2.5 m	10 m	1.5m
G14	7.5 m	3.5 m	9 m	2 m
G17	8 m	2.5 m	8 m	2 m
G22	9 m	0 m	12 m	0 m

4 Key: m - microcolonies present within zone of inhibition

5

6 Conclusions

7

8 Zone sizes were similar to the results from Example 2.
 9 Control suture G22 again demonstrated activity against
 10 both enterococci and Gp B streptococci. The zone sizes
 11 for the E coli using a lighter inoculum were similar to
 12 previous results.

13

14 EXAMPLE 4: Controlled Release

15

16 Suture Material

17

18 G11 - previously noted to yield a small zone of
 19 inhibition with NCTC 11047.

1 G16 - previously noted to yield a large zone of
2 inhibition with NCTC 11047.

3

4 Test organism

5

6 *Staphylococcus epidermidis* NCTC 11047.

7

8 Method

9

10 A single plate of Oxoid Iso-sensitest agar (Plate 1)
11 was seeded with the test organism to achieve a semi-
12 confluent growth. Four G11 sutures were applied to one
13 side of the plate, with four G16 sutures on the
14 opposite side. Each suture had been bent to yield a 90°
15 kink in the middle. After 24 hours incubation at 37°C
16 the zones of inhibition at right angles to the sutures
17 were recorded and the sutures were transferred to a
18 freshly seeded Iso-sensitest plate (Plate 2). The kink
19 in the suture ensured that the same aspect of the
20 suture was in contact with the agar surface on each
21 occasion. The new plate was incubated for a further 24
22 hours and the sutures were removed prior to assessment
23 of zones of inhibition.

24

25 Results

26

27 Plate 1 - Each of the G11 sutures yielded a zone of
28 inhibition 1.5 mm in (maximum) width. The G16 sutures
29 yielded zones 2.0 mm in width.

30

1 Plate 2 - After transfer to Plate 2, zones of
2 inhibition were not seen for either suture. On removal
3 of the sutures it was observed that there was confluent
4 growth of the test organism under the G11 sutures, but
5 there was inhibition of growth under G16.

6

7 Conclusions

8

9 After 24 hours in contact with the agar surface of
10 Plate 1, suture G11 had no demonstrable activity
11 against the test organism on Plate 2. Suture G16
12 demonstrated marginal activity on Plate 2, with
13 inhibition of growth directly underneath the suture
14 material.

15

16 EXAMPLE 5: Controlled Release

17

18 Suture material

19

20 G15 - previously noted to yield a large zone of
21 inhibition with NCTC 11047.

22

23 Test organism

24

25 *Staphylococcus epidermidis* NCTC 11047.

26 Method

27

28 A single plate of Oxoid Iso-sensitest agar was seeded
29 to yield a semi-confluent growth of NCTC 11047.

30 Sixteen sutures were applied with sterile forceps and
31 the plate was incubated at 37°C. At various time

1 intervals sutures were removed. Two sutures were
2 assessed for each time except for "24 hours" where 4
3 sutures were used. At the end of the 24-hour period
4 the zones of inhibition were assessed and the plate was
5 photographed.

6

7 Results and Conclusions

8

9 Suture G15 exhibited activity against the test organism
10 when in contact with the agar surface for only 5
11 minutes. Activity increases up to the 3 hour point,
12 after which no increased activity is seen.

13

14 EXAMPLE 6: Duration of Anti-microbial effect

15

16 Suture

17

18 G16.

19

20 Test organism

21

22 *Staphylococcus epidermidis* NCTC 11047.

23

24 Method

25

26 An Oxoid Iso-sensitest agar plate was seeded with the
27 test organism to achieve a semi-confluent growth. Six
28 G16 sutures were applied with sterile forceps and the
29 plate was incubated for 24 hours at 37°C. An
30 uninoculated Iso-sensitest plate was also incubated as
31 the Control.

1 After the initial incubation period each suture was
2 surrounded by a zone of inhibition. Three of the six
3 sutures were then removed. Each zone of inhibition was
4 challenged using a calibrated loop to apply a drop of
5 standardised suspension of test organism. Each drop
6 contained approximately 10^4 colony forming units. An
7 identical drop was applied to the Control plate. Both
8 plates were then incubated for a further 24 hours and
9 the zones were challenged again, and a further drop was
10 added to the Control plate. The procedure was repeated
11 on a daily basis. The end point of the experiment was
12 when growth appeared in the original zones of
13 inhibition following challenge, or when the Control
14 plate lost the ability to support organism growth due
15 to progressive dehydration. (This was minimised by
16 incubating the plates in an atmosphere with high
17 humidity.)

18

19 Results

20

21 Over the thirteen days of the experiment, no growth was
22 seen in any of the zones of inhibition. There was no
23 difference between the zones where the suture remained
24 in place and the zones where the suture had been
25 removed. The experiment was terminated at the 13 day
26 point even though the Control plate continued to
27 support growth of the challenge organism. This was
28 because the test plate appeared to be dehydrating more
29 rapidly, presumably because of the influence of the
30 lawn of growth of NCTC 11047 on its surface.

31

1 Conclusions

2

3 Experiment 1 demonstrated that much of the activity of
4 the suture is released in the first 24 hours.

5 Experiment 2 showed that activity is present within 5
6 minutes of contact with the agar. Experiment 3

7 illustrates that even though the suture may be

8 depleted, the surrounding area returns antimicrobial
9 activity over a period in excess of one week.

11

12

15 1. Objective

14

15 To determine the cytotoxicity of a series of suture
16 samples using a standard extraction/elution test, after
17 ISO 10993 part 5.

18

19 2. Scope

20

21 The test procedure applies to all suture samples which
22 were received sterile.

23

24.3. Equipment and Materials

25 3.1 Equipment

3.1.1 Laminar air flow hood

27 3.1.2 Incubator maintained at 37°C/5% carbon
28 dioxide.

29 3.1.3 Refrigerator at 4°C

30 3 3 4 Freezer at -18°C

3.1.5. Vacuum pressure

1 3.1.6 Phase contrast microscope.

2

3 3.2 Materials

4 3.2.1 Sterile plastic-ware pipettes.

5 3.2.2 Sterile glass pipettes.

6 3.2.3 24 well sterile dishes.

7 3.2.4 Surgical grade forceps.

8 3.2.5 Surgical grade scissors.

9 3.2.6 Sterile Universal containers.

10 3.2.7 L929 cell culture line (ATCC NCTC Clone
11 929).

12 3.2.8 TCPS negative control.

13 3.2.9 Natural rubber latex control.

14 3.2.10 Other control samples were supplied in
15 suture form.

16

17 4. Procedure

18

19 4.1 Test sample preparation

20 4.1.1 Test samples and controls were cut to
21 the appropriate size (see Section
22 4.2.1).

23 4.1.2 Tissue culture polystyrene was employed
24 as a negative control. Natural rubber
25 latex was employed as a positive
26 control. The controls were not in the
27 same physical form as the test material.

28

29 4.2 Extraction/elution method

30

1 All procedures carried out within laminar air
2 flow.

3 4.2.1 Sutures were prepared to provide a
4 surface area equivalent to 120 cm sq.
5 for each 20 mL of extracting medium.

6 4.2.2 Suture samples (typically 6 cm in
7 length) were transferred to Sterile
8 Universal containers.

9 4.2.3 Each container was labelled with the
10 test material code number.

11 4.2.4 20 mL of mammalian cell culture medium
12 (199) was added to each container.

13 4.2.5 The containers were placed in the
14 incubator 37°C/5% carbon dioxide for 24
15 hours.

16

17 4.3 Cell preparation

18 4.3.1 A cell subculture was prepared on the
19 same day the extracts were initiated.

20 4.3.2 Cells were plated into 24 well dishes at
21 a cell concentration of approximately 1
22 x 10⁵ cells mL. Enough wells were
23 prepared to allow four wells per test
24 sample. 2 mL of serum supplemented
25 medium was added to each well.

26 4.3.3 The 24-well plates were incubated for 24
27 hours at 37°C/5% carbon dioxide.

28

29 4.4 Test procedure

30 4.4.1 After 24 hours all 24 well plates were
31 examined by phase-contrast microscope

(x20 objective lens) to ensure healthy monolayer of >80% confluence.

3 4.4.2 The culture medium is aspirated.

4 4.4.3 The Universal containers are removed
5 from the extraction conditions, the pH
6 monitored using phenol red indicator.

7 4.4.4 2 mL of extracted medium is placed in
8 each well and the plates re-incubated
9 for a 48-hour period.

10

11 4.5 Interpretation of results

12 4.5.1 At the conclusion of the incubation
13 period the plates are removed from the
14 incubator and examined under phase
15 contrast microscope using x10 and x20
16 objective lenses.

Reactivity Response Table

Reactivity Response Table		
Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	No more than 20% of the cells are round, loosely attached and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	No more than 50% of the cells are round and devoid of intracytoplasmic granules; extensive cell lysis and empty areas between cells
3	Moderate	No more than 70% of the cell layers contain rounded cells and/or are lysed
4	Severe	Nearly complete destruction of the cell layers

1 4.6 Results

2

3 The following table (Table 4) highlights the
4 results obtained following two separate tests:
5 Two readings were taken at each test. In all
6 cases negative control (TCPS) provided a 0 grade
7 and positive control provided a 2 grade.

8

9 **Table 4**

Material Code	Grade Test 1		Test 2		Material Code	Grade Test 1		Test 2		Material Code	Grade Test 1		Test 2	
G1	0	0	0	0	G9	0	0	0	0	G17	1	0	0	0
G2	0	0	0	0	G10	0	0	0	0	G18	1	0	0	0
G3	0	0	0	0	G11	0	0	0	0	G19	1	1	0	0
G4	0	0	0	0	G12	0	1	0	0	G20	1	1	0	0
G5	0	0	0	0	G13	1	1	0	0	G21	1	1	0	0
G6	0	1	0	0	G14	1	1	0	0	G22	2	1	0	1
G7	0	0	0	0	G15	1	1	0	0	G23	1	1	0	0
G8	0	0	0	0	G16	1	1	0	0					

10

11 Comments

12

13 The results as detailed provide a very subjective
14 assessment of material cytotoxicity. Where a grade 0 is
15 shown, there was no evidence of toxicity and a
16 confluent healthy monolayer of cells was present.
17 Where there was any evidence of floating cells or
18 morphological abnormality or sub-confluent growth a
19 grade 1 was allocated. It should be noted that
20 floating cells do not necessarily indicate toxicity.
21 It should also be noted that the test 2 indicated less
22 evidence of toxicity than test 1. The extracts (with

- 1 suture material removed) had been maintained in a
- 2 frozen state for 72 hours before re-testing.

**TABLE 2: Maximum width of zone of inhibition measured
at right angles to the suture (millimetres)**

ORGANISM						
	1 Enterococcus	2 Staphylococcus	3 VRE	4 MRSA	5 Staph 11047	6 Gp B Strep
Suture						
G1	6m (7m)	0 (0)	8 (9)	0 (1)	1 (0)	9 (8)
G2	3.5m	0	3	1	1	1.5
G3	5m	1	7	1.5	1	7
G4	7m	1	8.5	1.5	1.5	7
G5	3.5m	1	7.5	1.5	1.5	9
G6	5m	2	8	1.5	1.5	3
G7	6.5m	2	8	1.5	2	7
G8	6.5m	2	8	2	2.5	8
G9	7m	2	9	1.5	1.5	8
G10	7m	2	8	1	1.5	7
G11	6m	2m	8	2m	1.5	7
G12	2m	2m	7	2m	2	8
G13	5m	2.5m	7.5	2m	1.5m	8
G14	6m	2.5m	7	1.5m	2.5m	3.5
G15	6m	2.5m	8	2m	2.5	8.5
G16	7m	2.5m	8	2.5m	2.5	8.5
G17	5.5m	2	9	1.5	2	7.5
G18	7m	2	9	1.5	1.5	7
G19	8m	1.5	12	1.5	1.5	8
G20	8m	1.5	13	1	1.5	8
G21	1.5m	2m	2	2m	1.5	2.5
G22	8m	0	9	0	0	12
Control						
G23	0	0	0	0	0	0

**TABLE 2 (CONT'D): Maximum width of zone of inhibition
measured at right angles to the suture (millimetres)**

ORGANISM						
	7 <i>Steno Malto</i>	8 <i>Pyo 1</i>	9 <i>Pyo 2</i>	10 <i>Serr marcescens</i>	11 <i>Enter cloacae</i>	12 <i>Morg morganii</i>
Suture						
G1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
G2	0	0	1	0	0	0
G3	0	1	1	0	0	1
G4	0	1	1	1	0	0
G5	0	1	1.5	1	0	1
G6	0	1.5	1.5	1.5	0	1.5
G7	0	1.5	2	1.5	1	2
G8	0	1.5	2	1.5	0	2
G9	0	1	1	1.5	0	1.5
G10	0	1	1	1.5	0	1.5
G11	0	0	1	1.5	0	1.5
G12	0	0	1.5	1.5	0	0
G13	1	2	1.5	1	0	2
G14	1	0	2	2	1	1
G15	1	2	2	2	1	2.5
G16	1	1.5	2.5	2	1	2.5
G17	1	1	1.5	1.5	1	2
G18	0	0	1.5	1	0	1.5
G19	0	0	1.5	0	0	1.5
G20	0	1	1	1	0	1
G21	1	1.5	1	1.5	1	2
G22	0	0	0	0	0	0
Control						
G23	0	0	0	0	0	0
Control						

**TABLE 2 (CONT'D) : Maximum width of zone of inhibition
measured at right angles to the suture (millimetres)**

	ORGANISM				
	13 <i>E coli</i>	14 <i>Kl pneumoniae</i>	15 <i>Acinetobacter sp</i>	16 <i>C albicans</i>	17 <i>C glabrata</i>
Suture					
G1	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
G2	0	0	0	0	0
G3	0	1	0	0	0
G4	1	1	0	0	0
G5	1	1	1	0	0
G6	1.5	1.5	1.5	0	0
G7	1.5	1.5	0	0	0
G8	1.5	1.5	1.5	0	0
G9	1	1	1	0	0
G10	1.5	1	1	0	0
G11	1.5	1.5	1	0	0
G12	1	1	1	0	0
G13	1.5	2m	1.5m	0	0
G14	2m	2m	2m	0	0
G15	2m	2m	2m	0	0
G16	2m	2m	2m	0	0
G17	1.5	1.5	1.5	0	0
G18	1	1	1.5	0	0
G19	0	1	1.5	0	0
G20	1.5	1	1	0	0
G21	1.5	1.5	1.5m	0	1
G22	0	0	0	0	0
Control					
G23	0	0	0	0	0
Control					

Key :

() - G1 Replicate result

m - microcolonies present within zone of inhibition

1 **Claims :**

2

3 1. A surgical suture material having either:

4

5 a) an external surface at least partially coated
6 with an anti-microbial composition comprising
7 an anti-microbial agent; or
8 b) an anti-microbial agent incorporated therein.

9

10 2. The suture material as in Claim 1, wherein said
11 material is selected from silk, polyester, nylon,
12 polypropylene, polyvinylidenefluoride, linen,
13 steel wire, catgut, polyglycolactide, polyamide,
14 fibroin, polyglycolic acid and copolymers thereof.

15

16 3. The suture material as claimed in Claim 1 or 2,
17 wherein said material is selected from
18 monofilament, braided multi-filament and twisted
19 multifilament yarns.

20

21 4. The suture material as claimed in any one of
22 Claims 1 to 3, wherein said anti-microbial
23 composition is coated on the suture surface.

24

25 5. The suture material as claimed in any one of
26 Claims 1 to 4, wherein said anti-microbial agent
27 is biodegradable.

28

29 6. The suture material as claimed in any one of
30 Claims 1 to 5, wherein said anti-microbial agent
31 is admixed with a coating material.

- 1 7. The suture material as claimed in any one of
- 2 Claims 1 to 6, wherein said anti-microbial agent
- 3 is a water-soluble metal ion-releasing glass.
- 4
- 5 8. The suture material as claimed in Claim 7, wherein
- 6 said glass is in particle form.
- 7
- 8 9. The suture material as claimed in Claim 7 or 8,
- 9 wherein said glass enables controlled release of
- 10 metal ions selected from Ag, Zn, Cu, Mg, Ce, Mn,
- 11 Bi, Se, Cs and mixtures thereof.
- 12
- 13 10. The suture material as claimed in Claim 9, wherein
- 14 said glass enables controlled release of metal
- 15 ions selected from Ag, Cu, Zn, Mg and mixtures
- 16 thereof.
- 17
- 18 11. The suture material as claimed in any one of
- 19 Claims 7 to 10, wherein said glass releases silver
- 20 ions.
- 21
- 22 12. The suture material as claimed in any one of
- 23 Claims 7 to 11, wherein said glass comprises a
- 24 source of silver ions which is introduced during
- 25 manufacture as silver orthophosphate (Ag_3PO_4).
- 26
- 27 13. The suture material as claimed in any one of
- 28 Claims 7 to 12, wherein said glass comprises up to
- 29 5 mole % of silver.
- 30

1 14. The suture material as claimed in any one of
2 Claims 7 to 13, wherein said glass comprises
3 phosphorous pentoxide (P_2O_5) as a glass former.

4

5 15. The suture material as claimed in Claim 14,
6 wherein the mole percentage of phosphorous
7 pentoxide in the glass composition is less than
8 85%, preferably less than 60% and especially
9 between 30 and 60%.

10

11 16. The suture material as claimed in any one of
12 Claims 7 to 15, wherein said glass comprises a
13 glass modifier selected from alkali metals,
14 alkaline earth metals, lanthanoid oxides,
15 lanthanoid carbonates and mixtures thereof.

16

17 17. The suture material as claimed in any one of
18 Claims 14 to 16, wherein said glass comprises a
19 glass modifier selected from sodium oxide (Na_2O),
20 potassium oxide (K_2O), magnesium oxide (MgO), zinc
21 oxide (ZnO), calcium oxide (CaO) and mixtures
22 thereof.

23

24 18. The suture material as claimed in Claims 16 or 17,
25 wherein the mole percentage of said glass modifier
26 is less than 60%, preferably between 40 and 60%.

27

28 19. The suture material as claimed in any one of
29 Claims 7 to 18, wherein said glass comprises a
30 boron containing compound.

31

- 1 20. The suture material as claimed in Claim 19,
2 wherein the mole percentage of said boron
3 containing compound is less than 15%, preferably
4 less than 5%.
- 5
- 6 21. The suture material as claimed in any one of
7 Claims 7 to 20, wherein said glass comprises an
8 additive compound selected from SiO_2 , Al_2O_3 , SO_3 ,
9 sulphate ions (SO_4^{2-}), transition metal compounds
10 and mixtures thereof.
- 11
- 12 22. The suture material as claimed in any one of
13 Claims 7 to 21, wherein said glass has a
14 dissolution rate in water at 38°C in the range from
15 substantially zero to 25mg/cm²/hour.
- 16
- 17 23. The suture material claimed in Claim 22, wherein
18 said dissolution rate is in the range from 0.01 to
19 2.0 mg/cm²/hour.
- 20
- 21 24. The suture material as claimed in any one of
22 Claims 7 to 23 wherein said glass comprises 20-35
23 mole % Na_2O , 18-30 mole % CaO and 45-60 mole %
24 P_2O_5 .

1 / 6

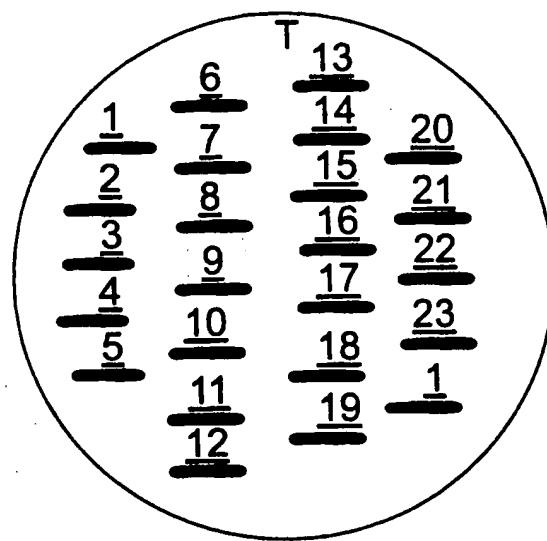
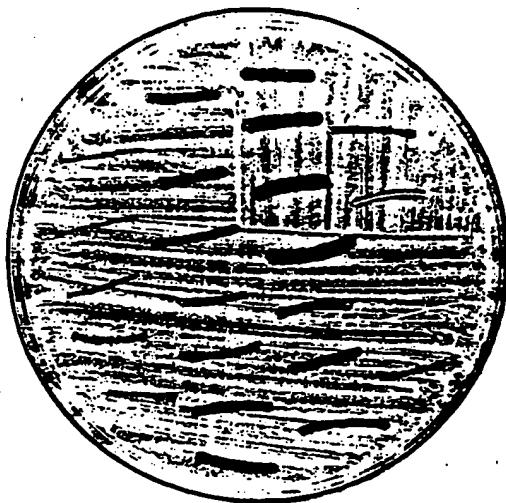


Fig. 1

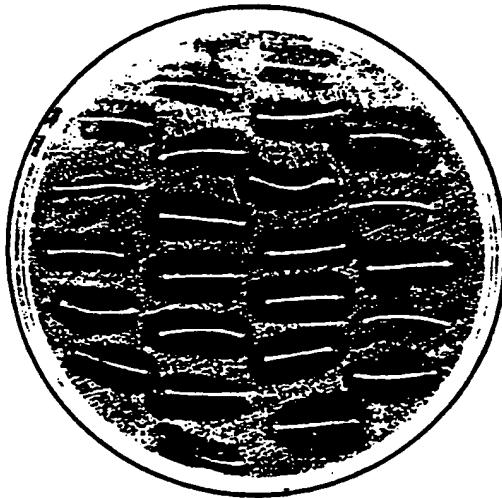
2 / 6



Enterococcus faecalis



Staphylococcus aureus



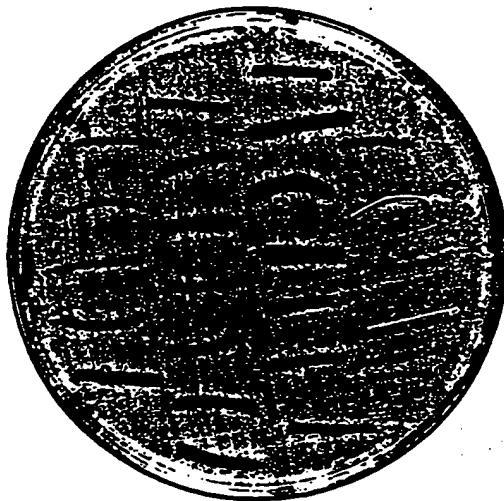
VRE



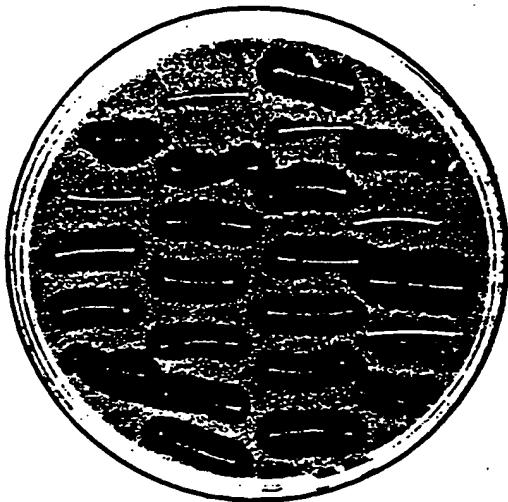
MRSA

Fig. 2

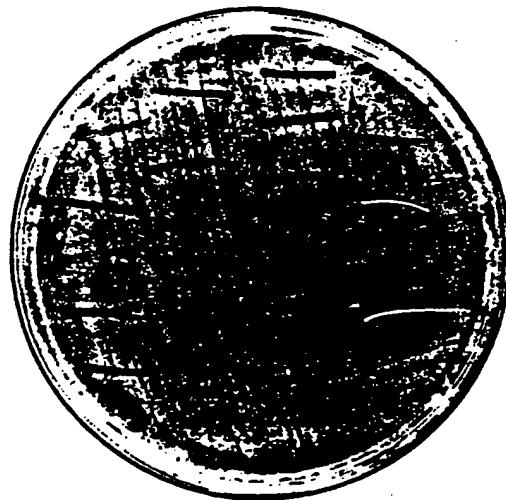
3 / 6



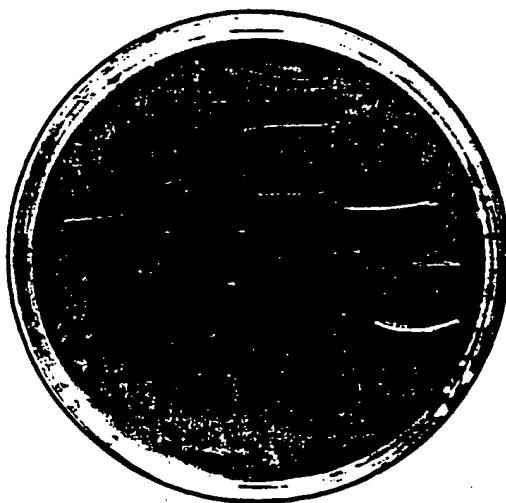
Staphylococcus epidermidis
NCTC 11047



Streptococcus agalactiae



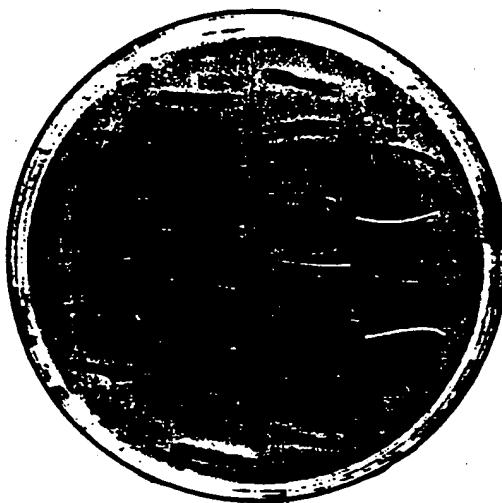
Stenotrophomonas maltophilia



Pseudomonas aeruginosa -
strain 1

Fig. 3

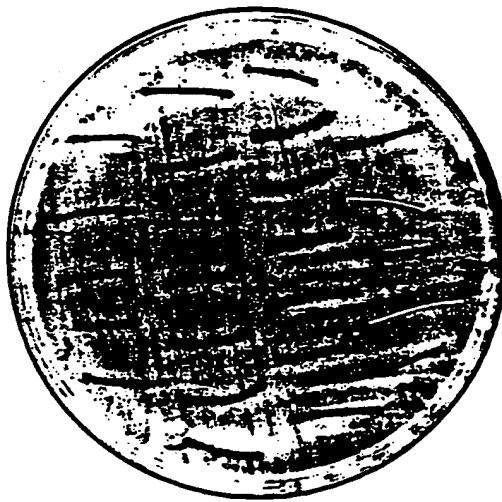
4 / 6



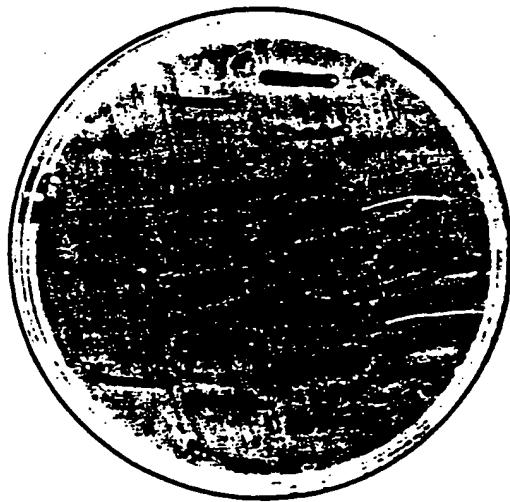
Pseudomonas aeruginosa -
strain 2



Serratia marcescens



Enterobacter cloacae



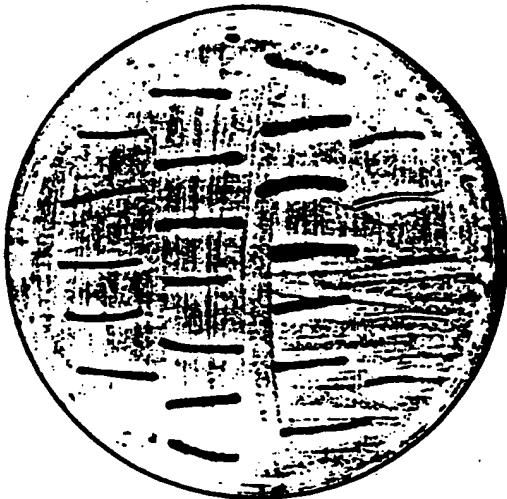
Morganella morganii

Fig. 4

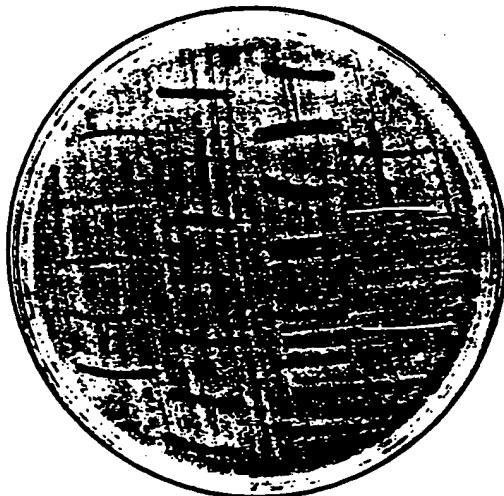
5 / 6



Escherichia coli



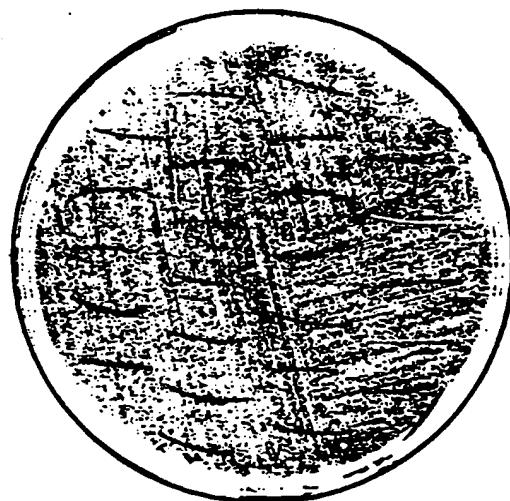
Klebsiella pneumoniae



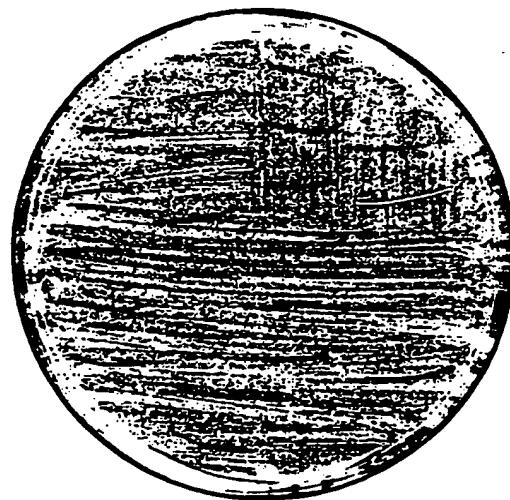
Acinetobacter sp

Fig. 5

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Candida albicans



Candida glabrata

Fig. 6

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/04049

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61L17/00 A61B17/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 A61L A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 633 032 A (SUMITOMO ELECTRIC INDUSTRIES, LTD.) 11 January 1995 (1995-01-11) the whole document	1-11
X	EP 0 328 421 A (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 16 August 1989 (1989-08-16) the whole document	1-11
X	US 5 413 788 A (EDWARDS ET AL.) 9 May 1995 (1995-05-09) abstract column 1, line 5-15 column 3, line 40-63 column 5, line 44-51 column 5, line 61 -column 6, line 31	1, 4, 6-13, 21

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

Date of mailing of the international search report

25 January 2001

31/01/2001

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European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Giménez Burgos, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/04049

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5 534 288 A (GRUSKIN ET AL.) 9 July 1996 (1996-07-09) the whole document -----	1-3,6-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/04049

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